

Phosphodiesterase 1C (PDE1C) ACTone™ Stable Cell Line

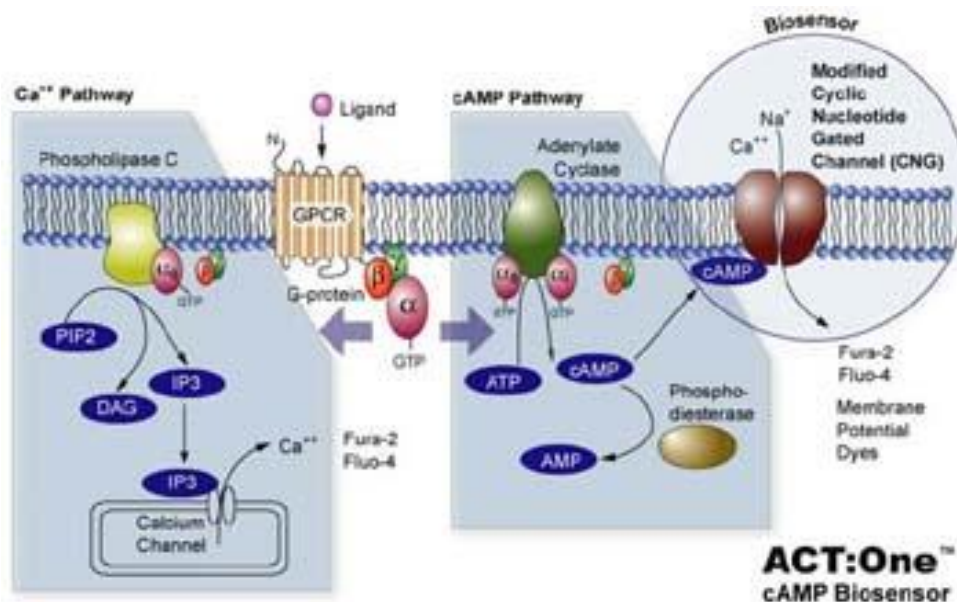
CATALOG NUMBER: CL-03-PDE1C

Introduction

The protein encoded by PDE1C gene belongs to the cyclic nucleotide phosphodiesterase (PDE) family, and PDE1 subfamily. Members of the PDE1 family are calmodulin-dependent PDEs that are stimulated by a calcium-calmodulin complex. This PDE has dual-specificity for the second messengers, cAMP and cGMP, with a preference for cGMP as a substrate. cAMP and cGMP function as key regulators of many important physiological processes.

Description

Human PDE1C ACTone™ is a HEK293-CNG-Gs cell line that expresses human PDE1C. HEK293-CNG-Gs cells express a modified CNG (Cyclic Nucleotide Gated) channel that opens in response to elevated intracellular cAMP levels and consequently result in ion flux (often detectable by calcium-responsive dye, Cat# CA-C155) and cell membrane depolarization which can be easily measured with fluorescent Membrane Potential Dye (Cat# CA-M165). The assay allows both end-point and kinetic measurement of intracellular cAMP changes with a FLIPR, or a fluorescence microplate reader.



Parental Cells

HEK-293 CNG-Gs cells (originally developed by BD Biosciences by introducing Gs-GPCR in HEK-293 CNG cells) (Cat# CL-03-PC10)

Gene/Enzyme Introduced

PDE1B (Genbank Accession No. NP_005011.1)

Applications

- cAMP dependent human PDE1C cell based assay
- cell based high-throughput screening of human PDE1B inhibitors

Functional Test

- this cell line has been tested positive for PDE1B specific response
- surviving rate: More than 2.5 million/vial on the second day after thawing
- the receptor specific activity is stable for 10 weeks continuous passage

Mycoplasma Contamination Test

This lot of cells has been tested and found to be free of mycoplasma contamination.

Content

- Stable cells: 1 mL (1×10^6 cells/mL in 70% DMEM, 20% FBS, 10% DMSO)

Growth Properties

Adherent

Cell Culture Medium

- DMEM-10% FBS supplemented with 250 μ g/ml G418, 1 μ g/ml Puromycin and 5 μ g/ml blasticidin.
- Freezing medium: 10% DMSO, 90% complete cell culture medium

Storage

Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C , preferably in liquid nitrogen vapor, until ready for use.

Data Analysis

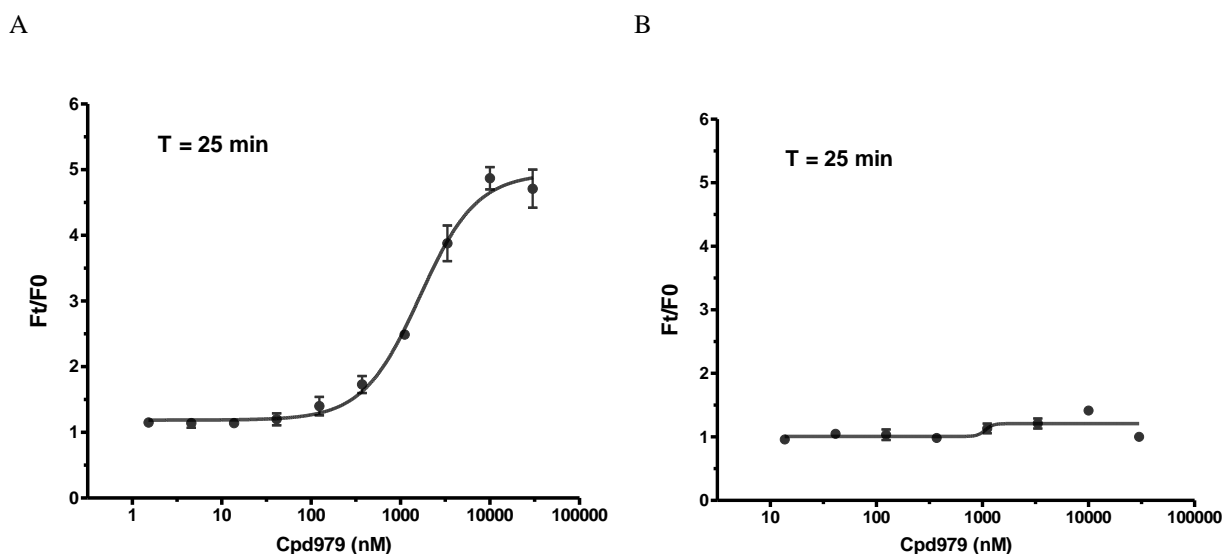


Figure 1. Response of ACTOne™ PDE1C cell line & parental cell line to Cpd979.

ACTOne™ PDE1C cells and parental cells (Cat# CL-03-PC10) were plated overnight in 20 μ l culture medium on a 384 well Biocoat plate. The next day, cells were dye-loaded with 20 μ l/well of membrane potential dye (CA-M165). After 2 hours of incubation at room temperature, baseline was recorded using a FlexStation (Molecular Devices) (F₀). 10 μ l of PDE inhibitors at various concentrations were added to the cell plate, and the data was recorded 25 minutes (F_t) after drug addition. Dose response curves were generated by Prism.

- Dose response curve of Cpd979 in ACTOne™ PDE1C cell line. IC₅₀ = 1.64 μ M in the presence of 10 μ M Ro 20-1724**
- Parental cells do not respond to Cpd979 in the presence of 10 μ M Ro 20-1724**



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